Stereodefined Phosphorothioate Analogues of DNA: Relative Thermodynamic Stability of the Model PS-DNA/DNA and PS-DNA/RNA Complexes†

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ABSTRACT: Thermodynamic data regarding the influence of P-chirality on stability of duplexes formed between phosphorothioate DNA oligonucleotides (of either stereo-defined all-R_P or all-S_P or random configuration at the P atoms) and complementary DNA or RNA strands are presented. Measured melting temperatures and calculated $\Delta G_{37}°$ values showed that duplexes formed by PS-DNA oligomers with DNA strands are less stable than their unmodified counterparts. However, relative stability of the duplexes ([all-R_P]-PS-DNA/DNA vs [all-S_P]-PS-DNA/DNA) depends on their sequential composition rather than on the absolute configuration of PS-oligos, contrary to the results of theoretical considerations and molecular modeling reported in the literature. On the other hand, for all six analyzed pairs of diastereomers, the [all-R_P]-PS isomers form more stable duplexes with RNA templates, but the origin of stereodifferentiation depends on the sequence with more favorable entropy and enthalpy factors which correlated with dT-rich and dA/dG-rich PS-oligomers, respectively.

Phosphorothioate analogues of oligodeoxyribonucleotides (PS-oligos) have played an important role for many years as molecular probes for investigation of biological processes and recently found new applications as antiviral and, potentially, anticancer therapeutics. Several important features make them interesting and useful: they are polyanionic species isoelectronic with natural DNA and selectively hybridize to complementary DNA and RNA sequences, are much more resistant toward inter- and intracellular phosphodiesterases, and, when complexed with RNA, are able to activate RNase H. However, replacement of sulfur for the nonbridging oxygen atom of the internucleotide phosphate moiety affects negative charge distribution within the phosphorothioate anion. Moreover, such replacement also creates a center of asymmetry at the phosphorus atom. The methods routinely used for chemical synthesis of PS-oligos provide a random mixture of diastereomers ([mix]-PS-oligos), while the enzymatic methods of synthesis provide isomers of R_P configuration, [all-R_P]-PS-oligos (*1*). Thus, data so far reported on thermodynamic stability of duplexes containing fully modified phosphorothioate strands were obtained only for such constructs (*2*). It should be emphasized that PS-oligos prepared by non-stereocontrolled methods consist of a mixture of 2^n diastereomers (n is the number of phosphorothioate linkages) and, in principle, each of them may interact with another chiral biomolecule in a slightly different way. Moreover, experiments on chimeric oligomers containing only every second phosphorothioate linkage of defined configuration indicated that the stability of the modified duplex depends on stereochemistry (*3*).

Theoretical considerations suggested that a duplex involving [all-S_P]-PS-oligos should be more stable than those consisting of [all-R_P]-PS counterparts. This prediction has been rationalized in terms of destabilizing interactions caused by the higher steric demands of the sulfur atom (as compared with the oxygen atom) directed "inward" to the B-type double helix formed with the [all-R_P]-PS oligomer (*4*). Furthermore, if the negative charge within a PS anion is localized predominantly at the sulfur atom (*5*), its inward orientation may cause stronger repulsion of negative charges present in complementary strands. These rather intuitive considerations were nevertheless supported by the results of molecular modeling (*6*), but until recently the lack of [all-S_P]-PS-oligomers hampered experimental verification of this hypothesis. Developed in the author's laboratory, the stereocontrolled method for synthesis of oligo(nucleoside phosphorothioate)s with P-stereodefined internucleotide phosphorothioate linkages allowed for studying of the thermodynamic stability of corresponding heteroduplexes at the molecular level.

In the present paper we report on the thermodynamic data regarding the influence of P chirality on the stability of duplexes formed between stereodefined PS-oligonucleotides and complementary DNA or RNA strands.

MATERIALS AND METHODS

Sodium chloride, sodium cacodylate, and Tris base (Aristar quality) were purchased from BDH Laboratory (Poole, England). Magnesium chloride pro analysi was obtained from Merck (Darmstadt, Germany). Hydrochloric acid (6 N) (amino acid analysis grade reagent) for the titration of Tris base was supplied by Applied Biosystems, Inc. (Foster City, CA). All absorption measurements were carried out in a 1

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Table 1: Melting Temperatures[a] and Standard Thermodynamic Parameters[b] of Association for PS-DNA/DNA Duplexes

group, sequence no.	DNA sequence	T_m (°C)				ΔG_{37}° (kcal/mol)			ΔS°[[cal/(mol·K)]			ΔH° (kcal/mol)		
		PO	mix	R_P	S_P	PO	R_P	S_P	PO	R_P	S_P	PO	R_P	S_P
I, **1**	AAAAAAAAAAAA	37	31	29	34	−7.9	−6.2	−7.4	−258	−232	−214	−87.8	−78.2	−73.2
I, **2**	GAGGAAAAAGAG	48	43	42	45	−11.5	−9.9	−10.1	−269	−248	−210	−95.0	−86.8	−75.1
I, **3**	GAGAGAAAAGAG	47	42	41	44	−10.9	−9.4	−9.9	−258	−234	−206	−82.2	−81.8	−73.7
I, **4**	GAGAGGAAAGAG	50	46	43	47	−11.7	−9.9	−10.5	−240	−214	−195	−86.1	−76.1	−71.1
II, **5**	TTTTTTTTTTTT	37	17	18	14	−7.9	−4.5	−3.5	−258	−156	−171	−87.8	−52.9	−56.4
III, **6**	CCTATAATCC	nd	nd	27	27	nd	−5.8	−5.8	nd	−169	−196	nd	−58.2	−66.6
III, **7**	AGATGTTTGAGCTCT	61	52	52	52	−16.2	−11.9	−12.1	−313	−226	−258	−113.3	−82.0	−92.1
IV, **8**	$G_RC_SA_RT_SA_RC_SA_RT_SG_RT$			34				−7.1			−168			−59.2
IV, **9**	$G_SC_RA_ST_RA_SC_RA_ST_RG_ST$			34				−7.4			−180			−63.2

[a] Melting curves for sequences **1** and **5**−**7** were recorded in 10 mM sodium cacodylate pH 7.4, 10 mM $MgCl_2$, and 70 mM NaCl, whereas for **2**−**4**, **8**, and **9** in 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 100 mM NaCl; the temperature gradient was 0.2 °C/min. The error of the temperature determination by the first derivative method did not exceed 1 °C. Total strand concentrations were as follows: **1** and **5**, 4.8 μM; **2**−**4** and **6**, 4.0 μM; **7**, 6.6 μM; **8** and **9**, 7.48 μM. [b] Standard errors of the fitting procedure did not exceed 1%.

cm path-length cell with a UV/Vis 916 spectrophotometer equipped with a Peltier thermocell (GBC, Dandenong, Australia).

Chemical Synthesis of Oligonucleotides. The synthesis of stereo-defined PS-oligonucleotides was performed manually. The first nucleoside units were anchored to the solid support by a sarcosinyl linker (*7*). Appropriately protected deoxyribonucleoside monomers possessing a 3′-*O*-(2-thiono-4,4-"spiro"-pentamethylene-1,3,2-oxathiaphospholane) moiety were synthesized and separated chromatographically into pure diastereomers. The protocol for the synthesis has been published elsewhere (*8*). The synthesis of unmodified DNA and RNA oligonucleotides was performed on an ABI 380B DNA synthesizer (Applied Biosystems, Inc.) at a 1 μmol scale using standard phosphoramidite DNA and RNA protocols. All synthesized oligomers were purified by two-step RP-HPLC (DMT-on and DMT-off), and their purity was assessed by MALDI-TOF mass spectrometry and polyacrylamide gel electrophoresis.

Sample Preparation and Melting Profile Recording. The concentration of oligomers was determined spectrophotometrically by UV absorbance at λ_{max} in water, using the extinction coefficients calculated by the published method (*9*). The samples were then lyophilized and redissolved in 10 mM Tris-HCl or sodium cacodylate, 100 mM NaCl, and 10 mM $MgCl_2$ buffer (pH 7.4). Melting profiles were measured with a temperature gradient of 0.2 °C/min in both directions to check whether observed transitions were reversible. The melting temperatures were calculated using the first-order derivative method.

CD Spectra Recording. CD spectra were recorded on a CD6 dichrograph (Jobin-Yvon, Longjumeau, France) using cells with 5 mm path length, 2 nm bandwidth, and 1−2 s integration time. Each spectrum was smoothed with a 9- or 15-point algorithm (included in the manufacturer's software, version 2.2.1) after averaging at least three scans.

Thermodynamic Parameter Calculation. Thermodynamic parameters were obtained numerically by fitting an analytical curve resulting from a two-state model to experimental melting profiles. Equations for the two-state model were used as derived by Evertsz et al. (*10*). The procedure applied for fitting was a nonlinear least-squares routine included in SigmaPlot software (version 5.01, Jandel Corp.).

RESULTS

Thermodynamic Properties of PS-DNA/DNA Duplexes. The presently studied oligomers can be divided into four groups as listed in Table 1. The first group consists of homopurine dodecamers **1**−**4**. The second group consists of only a single homothymidylate dodecamer **5**, while there are two mixed sequences of different length (**6** and **7**) in group III. All oligomers in groups I−III were prepared as random mixtures of diastereomers ([mix]-PS) as well as [all-R_P]-PS and [all-S_P]-PS stereodefined diastereomers. Group IV consists of two isosequential decamers (**8** and **9**) with alternate predetermined absolute configurations at phosphorus atoms at consecutive centers. Notably, the corresponding phosphorothioate centers in **8** and **9** have opposite configurations. As reference compounds, corresponding unmodified oligonucleotides (PO-oligos) were also prepared. The thermodynamic parameters for complexes formed by oligomers **1**−**9** with complementary DNA strands (Table 1) were derived from recorded melting profiles. The absence of hysteresis on these profiles indicated that observed transitions are related to dissociation of double-stranded helices. Both measured T_m and calculated ΔG_{37}° values confirmed that all duplexes formed by phosphorothioate oligomers with DNA strands are less stable than their unmodified counterparts, but surprisingly, relative stability depends on their sequential composition rather than on the absolute configuration of PS-oligos present in the duplexes. For homopurine oligomers (group I) the [all-S_P]-PS isomers form duplexes more stable by 4−5 °C than their [all-R_P]-PS counterparts, contrary to the homothymidylate dodecamer **5**, where the duplex [all-R_P]-PS/DNA was more stable by 4 °C. This suggests that for mixed sequences, consisting of balanced purine and pyrimidine residues, the stereodifferentiation may be negligible and too low to be observed. The data found for phosphorothioates belonging to groups III and IV are consistent with this assumption, as there is no difference between corresponding duplexes in terms of T_m and ΔG_{37}°. To determine the possible origin of observed differences in stability in groups I and II, ΔH° and ΔS° values were derived numerically from melting curves. They show distinct regularities, as for the formation of all PS-DNA/DNA duplexes the observed changes of enthalpy and entropy were smaller, compared to values calculated for their unmodified counterparts. On the other hand, for the more stable modified duplexes of given sequences, i.e., for duplexes containing

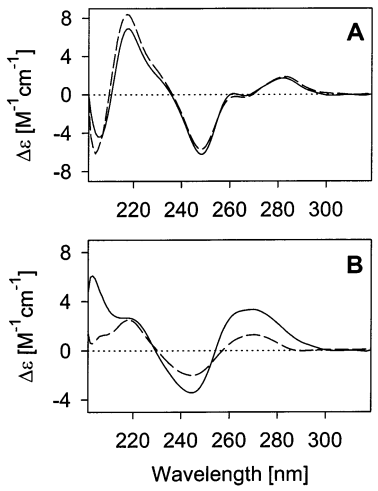

FIGURE 1: CD spectra of duplexes formed by [all-R_P]-PS (solid line) and [all-S_P]-PS (dashed line) oligomers with DNA matrices. Panel A: PS-**1**/DNA (74.4 μM per base) in 10 mM sodium cacodylate buffer (pH 7.4) with 10 mM $MgCl_2$ and 100 mM NaCl. Panel B: PS-**6**/DNA (110 μM per base) in the 10 mM Tris buffer (pH 7.4) with 10 mM $MgCl_2$ and 100 mM NaCl. All spectra were recorded at 5 °C.

[all-S_P]-PS isomers in group I and [all-R_P]-PS isomers in group II, the changes of both parameters were smaller than those determined for less stable species. Smaller $\Delta H°$ and $\Delta S°$ values found for [all-R_P]-PS isomers in group III and for the [R_P/S_P]-**8** do not correspond with thermal stability, as T_m and $\Delta G_{37}°$ parameters are virtually identical to those for [all-S_P]-PS forms and [S_P/R_P]-**9**, respectively.

The described regularities in thermodynamic properties do not correlate with conformational properties of the double-stranded helix, at least as judged from CD spectra (Figure 1). The most profound stereodifferentiation noticed in group I ($\Delta T_m = 5$ °C for PS-dA_{12}/T_{12}) is accompanied by very small differences in corresponding CD spectra (Figure 1A). Rather unexpectedly, significant differences were present in the CD spectra for PS-**6**/DNA (Figure 1B), for which no stereodifferentiation of thermal stability was found.

Thermodynamic Properties of PS-DNA/RNA Duplexes. The sequences of oligomers under study are listed in Table 2. Contrary to the properties of PS-DNA/DNA duplexes, regardless of the sequence of the phosphorothioate strands, the [all-R_P]-PS-DNA/RNA duplexes are thermally more stable than their [all-S_P]-PS-DNA/RNA counterparts; i.e., they have higher melting temperatures and lower $\Delta G_{37}°$ values. On the other hand, there are no apparent regularities for the corresponding $\Delta H°$ and $\Delta S°$ values. For sequences **7**, **10**, and **11**, the more stable modified duplexes consisting of [all-R_P]-PS isomers are formed with smaller change of both enthalpy and entropy, whereas for sequences **4**, **12**, and **13** the smaller changes accompany formation of the less stable duplexes with participation of [all-S_P]-PS diastereomers. However, similar to PS-DNA/DNA duplexes, the unmodified PO-DNA/RNA heteroduplexes are more stable than corresponding PS-DNA/RNA heteroduplexes, and their association results in greater change of both enthalpy and entropy.

DISCUSSION

The conditions we chose for our study of thermodynamic properties of duplexes formed with participation of stereodefined phosphorothioate oligomers (10 mM $MgCl_2$, 100 mM NaCl) are slightly different from those typically used in such experiments (1 M NaCl and no magnesium cations). Our selection (i.e., lowered NaCl concentration and the presence of Mg^{2+} cations) is closer to physiological conditions and may provide biologically more important data with respect to application of phosphorothioate oligomers as therapeutic agents. To obtain thermodynamic parameters, we used a two-state model (*11*) and a method of nonlinear least-squares fitting of the analytical form of a melting curve to an experimental one (*10*). We checked the validity of this model for short duplexes containing phosphorothioate strands by comparison of the values obtained by the numerical fitting with those derived from linear dependence of a reciprocal melting temperature (T_m^{-1}) on a natural logarithm of total strand concentration (ln c_T). Simultaneously, we found that the standard errors for values obtained by a single fitting procedure (to a single melting curve) did not exceed 1%, whereas the standard errors of average values obtained from five experiments (at different strand concentrations) did not exceed 4%. The data were published elsewhere (*8*). The standard errors of calculated parameters are relatively small; however, in the present analysis we focus on differences rather than on absolute values. Although it was known that substitution of natural internucleotide linkages by phosphorothioates decreases duplex stability, the origin of such a decrease remained unclear. The data reported by Clark et al. (*12*) indicated that this destabilization is entropy driven for PS-DNA/DNA duplexes but enthalpy driven for PS-DNA/RNA. However, the conclusions were based on the analysis of structures formed by [mix]-PS oligomers of only d/r$(AC)_{12}$ and d/r$(GT)_{12}$ sequences. We extended their work analyzing stereodefined and [mix]-PS oligomers of several different sequences (Tables 1 and 2). Our data suggest that the entropy factor is more favorable and the enthalpy factor is less favorable, respectively, for all PS-DNA/DNA and PS-DNA/RNA systems studied, as compared with their unmodified counterparts. Thus, the destabilization caused by the presence of a sulfur atom in the internucleotide linkages is enthalpy driven, regardless of the nature of the matrices. There are some results in the literature supporting this conclusion. A smaller change of enthalpy during association of a duplex means weaker hydrogen bonds within complementary strands and/or weaker stacking interactions. The former is supported by the results of NMR studies on the stability of the homoduplex formed by Dickerson's decamer (*13*), which show that an average "lifetime" of complementary pairs is much shorter for the phosphorothioate oligomer (1−6 ms) than for its natural counterpart (14−30 ms). On the other hand, an NMR investigation of conformational changes of a double-stranded helix caused by consecutive phosphorothioate internucleotide linkages indicated that stacking interactions could be weaker at least in the PS-DNA/DNA duplex (*14*). Another premise indicating that the presence of sulfur atoms can disturb stacking interaction comes from our data. All three PS-dA_{12} oligomers (i.e., [mix], [R_P], and [S_P] forms), when associated with the T_{12} template, form much more stable duplexes than those composed of

Table 2: Melting Temperatures[a] and Standard Thermodynamic Parameters[b] of Association for PS-DNA/RNA Duplexes

		T_m (°C)				ΔG_{37}° (kcal/mol)			ΔS°[[cal/(mol·K)]			ΔH° (kcal/mol)		
sequence no.	DNA sequence	PO	mix	R_P	S_P	PO	R_P	S_P	PO	R_P	S_P	PO	R_P	S_P
4	GAGAGGAAAGAG	42	35	37	34	−9.6	−8.0	−7.2	−189	−182	−171	−68.2	−64.4	−60.2
7	AGATGTTTGAGCTCT	59	47	52	44	−14.1	−11.2	−9.4	−245	−170	−188	−90.0	−64.0	−67.6
10	TTTTTTTTTTTTTTTTTTT	44	24	29	20	−10.9	−6.9	−4.6	−266	−214	−232	−93.2	−73.3	−76.6
11	GCTATAATGG	nd	27	29	24	nd	−6.6	−5.6	nd	−183	−194	nd	−63.2	−65.6
12	CCACACCGACGGCGCCC	nd	71	74	69	nd	−20.6	−18.6	nd	−274	−267	nd	−105.7	−101.4
13	GAGGGCTGGAGAGCATC	70	61	64	59	−19.7	−17.3	−14.6	−301	−289	−246	−113.0	−107.7	−91.0

[a] Melting curves for all sequences but **4** were recorded in 10 mM sodium cacodylate, pH 7.4, 10 mM $MgCl_2$, and 70 mM NaCl, whereas for **4** in 10 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, and 100 mM NaCl; the temperature gradient was 0.2 °C/min. The error of temperature determination by the first derivative method did not exceed 1 °C. Total strand concentrations were as follows: **4** and **11**, 4.0 μM; **7** and **13**, 2.3 μM; **10** and **12**, 1.2 μM. [b] Standard errors of the fitting procedure did not exceed 1%.

any form of the PS-T_{12} and dA_{12} matrix (Table 1). This difference has enthalpic origin because for the formation of the latter duplexes the entropy factors are much more favorable. However, the pattern of hydrogen bonding between nucleobases is the same in both cases. On the other hand, it is known that stacking is more efficient for a homoadenylate strand than for a homothymidylate one. Thus, we suppose that phosphorothioate modification of internucleotide linkages results in a larger disturbance of stacking in a molecule with weaker interactions, resulting in a smaller change of enthalpy during duplex formation. In contrast to the changes of enthalpy, smaller changes of entropy during association are more favorable. It is the case for all studied duplexes containing a phosphorothioate strand. One of the possible reasons for such a situation can be a smaller difference in the hydration pattern between single- and double-stranded species involving phosphorothioate groups. The results of molecular modeling reported by Hartmann et al. (*15*) indicate that the ionic sulfur accessibility areas in the dsDNA are approximately twice as large as the ionic oxygen areas although the total sphere area of the sulfur is only 1.2 times larger, as compared to the total sphere area of the oxygen.

The above-discussed regularities are common for PS-DNA/DNA duplexes and PS-DNA/RNA heteroduplexes. However, the origin of stereodifferentiation seems to be different. Therefore, this problem will be discussed separately for both types of duplexes.

PS-DNA/DNA Duplex. Data presented in Table 1 show that the association of the most stable PS-DNA/DNA structure of given sequence ([all-S_P]-PS-**1**−**4**, [all-R_P]-PS-**5**) is connected with a more favorable entropy factor (the smaller change) and a more unfavorable enthalpy factor (the smaller change). Thus, stereodifferentiation of their stability is entropy driven. For more detailed analysis of this problem, we designed oligomers **8** and **9**. They were intended to form with complementary strands the duplexes with the same number of G-C and A-T base pairs as does oligomer **6** (four G-C and six A-T pairs). Notably, they have an alternating purine−pyrimidine sequence and an alternate configuration of internucleotide bonds. The duplexes PS-**8**/DNA and PS-**9**/DNA are equally stable (T_m = 34 °C; ΔG° = −7.1 and −7.4 kcal/mol), and their stability is higher than that for PS-**7**/DNA (T_m = 27 °C; ΔG° = −5.8 kcal/mol). Analysis of the thermodynamic parameters revealed that more favorable were ΔS° for oligomer **8** and ΔH° for **9**. Nonetheless, the remarkable stabilization of duplexes formed by **8** and **9**, compared to PS-**6**/DNA, is entropy driven because ΔH° = −59.2 and −63.2 kcal/mol for **8** and **9**, respectively, were less favorable than ΔH° = −66.6 kcal/mol found for **6**. Taking into account all regularities discussed above, we came to the conclusion that the presence of phosphorothioate groups of R_P configuration on the 5′- and/or 3′-side of pyrimidines is connected with favorable entropy factor. On the other hand, the higher stability of **8**/DNA compared to [all-R_P]-PS-**6**/DNA is mostly of enthalpic origin because the entropy components are virtually the same. More favorable enthalpy factors were also found for homopurine oligomers [all-R_P]-PS-**1**−**4** and for the homopyrimidine oligomer [all-S_P]-PS-**5**. Therefore, it seems that the presence of a phosphorothioate group of S_P configuration on the 5′- and/or 3′-side of pyrimidines correlates with a favorable enthalpy factor. If a pyrimidine nucleoside has on both sides phosphorothioate groups of opposite configuration, that on the 5′-side has a stronger effect on the thermodynamic parameters of the whole molecule; i.e., R_P-Py-S_P and S_P-Py-R_P motifs result in favorable changes of entropy and enthalpy, respectively. Such a conclusion can be drawn from the comparison of the appropriate parameters for the **9**/DNA and [all-R_P]-PS-**6**/DNA duplexes [ΔH° = −63.2 vs −58.2 kcal/mol whereas ΔS° = −180 vs −169 cal/(mol·K)]. Presented data do not allow for similar analysis in the case of purine nucleotides.

Our data obtained for PS-DNA/DNA duplexes challenge the hypothesis (*4*, *6*) that [all-R_P]-PS-oligos should form less stable structures than their [all-S_P]-PS counterparts for electrostatic or steric reasons. It seems that it is the sequence that influences the relative stability of structures formed by stereoregular isomers.

PS-DNA/RNA Duplex. Unlike PS-DNA/DNA duplexes, stereodifferentiation of the stability of PS-DNA/RNA heteroduplexes does not depend on their sequence; i.e., for all analyzed pairs of diastereomers the [all-R_P]-PS isomers form more stable structures (Table 2). The stereo differentiation is more distinct as ΔT_m's are in a range of 3−9 °C, compared with 0−5 °C observed for the PS-DNA/DNA duplexes. However, thermodynamic parameters do not allow one to draw conclusions about its origin. It seems that in the case of PS-DNA/RNA duplexes the origin of stereodifferentiation does depend on a sequence. In the case of oligomers containing relatively more thymidine residues (**7**, **10**, **11**) the higher stability of the structures formed by [all-R_P]-PS isomers comes from more favorable entropy factors [e.g., ΔS° = −170 vs −188 cal/(mol·K) whereas ΔH° =

−64.0 vs −67.6 kcal/mol for [all-R_P]-PS-**7**/RNA and [all-S_P]-PS-**7**/RNA, respectively]. If there are no (or few) thymidines in a given sequence (oligomers **4**, **12**, and **13**), the higher stability is determined by more favorable enthalpy factors. However, for a given sequence the relationship between $\Delta H°$ and $\Delta S°$ is the same regardless of the character of a template (e.g., PS-**7**/DNA and PS-**7**/RNA; Tables 1 and 2, respectively). These results suggest that the factors discussed for PS-DNA/DNA duplexes play a similar role for PS-DNA/RNA ones, but there is an additional, sequence-independent factor exclusively stabilizing structures formed by [all-R_P]-PS isomers with RNA templates. This can be particular hydration of the phosphate groups in a duplex existing in an A-type conformation. It is known that long-lived hydration patterns are present in the deep major groove of A-RNA (*16*) or A-DNA (*17*) and involve sequence-independent water bridges between *pro*-R_P oxygen atoms of adjacent phosphate groups. There are no such bridges in a case of B-DNA because corresponding distances are too long (*17*). Although DNA/RNA hybrids have less-defined hydration patterns than homogeneous duplexes (*18*), there is a tendency for a DNA strand to adopt the conformation of the RNA component, i.e., the A conformation. Therefore, adjacent phosphate groups of the DNA strand can be close enough to allow for formation of similar bridges. On the other hand, we have shown that the sulfur atom in a phosphorothioate group, carrying most of the negative charge of the internucleotide linkage, is a strong acceptor of the charge-assisted hydrogen bond (*19*). Simultaneously, a nonbridging oxygen in the same group is a much weaker acceptor because of significantly reduced charge, compared to the symmetrically distributed charge in the unmodified phosphate bond. Therefore, it is possible that described water bridges can be formed in the case of R_P isomers but not of S_P ones, thus constituting the specific stabilizing factor that does not exist for PS-DNA/DNA duplexes.

In general, presented data and their analysis indicate that the thermodynamic properties of duplexes formed by stereodefined phosphorothioate oligomers strongly depend on the nature of the matrix (DNA vs RNA).

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